WE CLAIM:

1. A process for preparation and purification of recombinant human IFN alpha 2b which comprises of :
   I. cultivating recombinant *Pichia pastoris* containing a hu-IFN alpha 2b gene.
   II. culturing said recombinant *Pichia pastoris* in complex/defined salt culture medium to produce hu-IFN alpha 2b protein.
   III. purifying recombinant hu-IFN alpha 2b protein from said culture medium
2. A process as claimed in claim 1 wherein said human IFN alpha 2b gene comprises of the following sequence:

Nucleotide sequence of recombinant human IFN alpha 2b gene:

SEQ ID 3:

TGT GAT CTG CCT CAA ACC CAC AGC CTG GGT AGC AGG AGG ACC TTG ATG
CTC CTG GCG CAG ATG AGG AGA ATC TCT CTT TTC TCC TGC TTG AAG GAC
AGA CAT GAC TTT GGA TTT CCC CAG GAG GAG TTT GGC AAC CAG TTC CAA
AAG GCT GAA ACC ATC CCT GTC CTC CAT GAG ATG ATC CAG CAG ATC TTC
AAC CTC TTC AGC ACA AAG GAC TCA TCT GCT GCT TGG GAT GAG ACC CTC
CTA GAC AAA TTC TAC ACT GAA CTC TAC CAG CAG CTG AAT GAC CTG GAA
GCC TGT GTG ATA CAG GGG GTG GGG GTG ACA GAG ACT CCC CTG ATG AAG
GAG GAC TCC ATT CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT CTC
TAT CTG AAA GAG AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT GTC AGA
GCA GAA ATC ATG AGA TCT TTT TCT TTG TCA ACA AAC TTG CAA GAA AGT
TTA AGA AGT AAG GAA TGA*.
(* = Stop codon.)

3. A process as claimed in Claim 1 wherein said recombinant *Pichia pastoris* containing a hu-IFN alpha 2b gene is cultivated by first isolating and purifying mRNA from human leucocytes, preparing a first strand of DNA from said purified mRNA to obtain said modified hu-IFN alpha 2b gene, amplifying said gene and cloning said amplified modified hu-IFN alpha 2b gene into an expression vector, , amplifying and isolating said hu-IFN alpha 2b gene from said modified interferon alpha 2b clone and cloning it into an expression vector and transforming it into said *Pichia pastoris.*
4. A process as claimed in claim 1 wherein said cloning is carried our by RT-PCR methods employing primer pairs having the sequence selected from: SEQ ID 4 & 5 ; 6 & 7; 8 & 9; 10 & 11; 12 & 13 and 12 & 14.
5. A process as claimed in Claim 3 wherein said host is Pichia
6. A process as claimed in Claim 3 wherein said vector is pPICZαA.
7. A process as claimed in Claim 6 wherein said IFN alpha 2b gene is cloned in pPICZαA vector down stream to AOX promoter and alpha mat signal sequence.

8. A process as claimed in Claim 3 wherein a desired construct containing IFN alpha 2b gene (expression cassette) is integrated in desired site of *Pichia* genome, at the AOX region.
9. A process as claimed in Claim 8 wherein said expression cassette is integrated at 5' AOX region of host *Pichia pastoris* selected from *Pichia pastoris* KM 71, *Pichia pastoris* KM 71H, *Pichia pastoris* GS115, *Pichia pastoris* X33 preferably *Pichia pastoris* KM71.
10. A process as claimed in Claim 9 wherein said *Pichia* has His auxotrophic phenotype.
11. A process as claimed in Claim 1 wherein said culture medium is selected from complex media like BGY, BY, BMY, BGYP, YPD, defined salt medium preferably defined salt medium and BMY.
12. A process as claimed in Claim 11 wherein said culture medium comprises a nitrogen source selected from one or more of ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, bean-cakes, potato extracts, protein hydrolysates, yeast extract, urea and ammonium hydroxide.
13. A process as claimed in Claim 1 or 11 wherein said culture medium comprises of a carbon source such as glycerol, glucose, fructose, methanol and the like, preferably glycerol.
14. A process as claimed in Claim 1, 11,12 or 13 wherein the biomass build up is in a range of from 35 to 100 g/L, preferably 35-50 g/L for complex media and 50 to 80 g/L, preferably 50-60 g/L for define salt media based on dry cell weight.
15. A process as claimed in claim 14 wherein said culture medium has:
    (a) pH in the range of 3.0 to 6.0, preferably 6.0 to 6.5 for complex media and 3.5 to 4.5 for defined salt media preferably 5.8 to 6.2.
    (b) temperature in the range of 25 to 35 °C preferably 28 to 32 °C.
    (c) dissolved oxygen: 20-80% of saturation, preferably 40-50% of saturation and said culturing is carried out for a duration of 48 to 110 hours, preferably 48 to 72 hours for complex media and 90-110 hours for defined salt media.
16. A process as claimed in Claims 11 to 15 wherein the expression of recombinant IFN alpha 2b protein is induced after reaching appropriate biomass buildup using suitable alcohol such as methanol, ethanol and the like preferably methanol at concentration of 0.1 to 3.0% v/v, preferably 1-1.5%v/v.
17. A process as claimed in Claim 16, wherein the expression of full length recombinant IFN alpha 2b protein is regulated by addition of nitrogen source selected from yeast nitrogen base, yeast nitrogen base without amino acid, yeast hydrolysate, yeast extract, peptone, casamino acid, meat extract, beef extract and like, preferably yeast extract and peptone along with or without propylene glycol.
18. A process as claimed in any preceding claim wherein said recombinant IFN alpha 2b protein is purified to homogeneity by

(a) separating the cells from the cell culture to obtaine the supernatant which contains expressed recombinant IFN alpha 2b protein.

(b) subjecting said supernatant to a cation exchange chromatography by

i) binding said expressed IFN on column packed with either CM sepharose FF, SP sepharose FF or sepraprep S

ii) washing said column with a buffer selected from citrate, phosphate,acetate buffer or CIEXI buffer, at a pH 5.0-5.5 to remove unwanted proteins.

iii) Eluting said IFN with CIEXII buffer with pH 4.8-5.4.

(c) Subjecting the eluent obtained in step b(iii) to anion exchange chromatography followed by elution with AIEX II buffer.

(d) Subjecting the eluent from step (c) to ultrafiltration using Amicon stirred cell with YM 10 membrane to obtain a concentrated retentate containing IFN protein;

(e) Subjecting said concentrated retentate to gel filtration chromatography using ammonium acetate buffer containing Tween-80 and EDTA, pH 5.2-5.5, to obtain homogenous species of IFN.

(f) purifying said IFN alpha 2b obtained in step (e) by repeating steps (a) to (e) in any sequence or order.

19. A pharmaceutical composition comprising of purified interferon alpha 2b according to claim 1 and 19, and a pharmaceutically acceptable carrier either in liquid form or in lyophilized form.

20. A pharmaceutically composition a claimed in Claim 19 wherein said pharmaceutically acceptable salt comprises phosphate buffer, glycine, HSA, PEG, ammonium acetate, NaCl, Tween-80, EDTA, Benzyl alcohol and the like in any combination and with desired concentration / amount.

21. A method of treatment and use of purified interferon alpha 2b of the present invention in the preparation of medicament for treatment of viral diseases like chronic active Hepatitis B, Chronic active Hepatitis non A-non B, Chronic active Hepatitis delta, Chronic active Hepatitis C; cancer diseases like Chronic myelogenous leukemia, Non-Hodgkin's lymphoma, AIDS related Kaposi's Sarcoma, Renal cell carcinoma, Malignant melanoma, Hairy cell leukemia, Bladder carcinoma, Superficial and noduloulcerative basal cell carcinoma, Condylomata acuminata, Laryngeal papillomatosis, and like.